THE BIOLOGICAL RELATIONSHIPS OF ASCARIDS

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The experiments described in this paper were undertaken with a view of determining whether, by means of immunological reactions, *Ascaris lumbricoides* which occurs in man can be differentiated from *Ascaris lumbricoides* which occurs in the hog. Morphologically, the forms from the two hosts are indistinguishable so far as present knowledge goes. The name *Ascaris suum* or *Ascaris suilla* which is used by certain writers to designate ascarids which occur in swine is not generally accepted by zoologists for the reason that the classification of animal parasites is based on morphology and not on host relationship. Despite the fact, however, that the specific identity of Ascaris from the hog and from man is commonly accepted on the basis of our present knowledge of the morphology of these forms, much work still remains to be done in order to establish that view beyond any doubt.

The problem which the present writer undertook to solve was whether the apparent morphological identity of *Ascaris lumbricoides* from man and from the hog is correlated with a biochemical identity so far as that can be determined by immunological tests. The solution of this problem necessitated preliminary information as to the possibility of differentiating genera and species of ascarids by immunological methods. The data presented in this paper cover several species of ascarids and throw light on the biological relationships of the forms under consideration.

Flury (1912) made a rather extensive study of the chemistry and toxicology of Ascaris and failed to find any essential differences between *Ascaris lumbricoides* and *Ascaris equorum,* two species that are quite distinct morphologically. Flury employed the methods of analytical chemistry and the usual technic of testing the physiological effects of tissue and organ extracts. The present writer resorted to the more delicate immunological tests by which specific differences may be more readily detected. The differentiation of the fluids of vertebrate species by means of immunological reactions has been studied by many investigators, notably by Nuttall and Uhlenhuth. The former (Nuttall, 1904) writes as follows with reference to the differentiation of the blood of vertebrates by means of cross-precipitin tests:

"The degree and rate of blood reaction appear to offer an index to the degree of blood relationship; in other words, closely related bloods react more powerfully (more precipitum) and more rapidly than do distantly related bloods, provided the latter react at all."

EXPERIMENTS WITH PRECIPITIN TESTS

As is well known the blood serum of an animal immunized to solutions containing proteins acquires the power of precipitating these proteins from solution. Rabbits are commonly used for the purpose of obtaining precipitating serum, and injections are made at intervals of about six days. Four or five injections are usually sufficient to produce a rich precipitin content in the serum.

Following the above technic the present writer immunized a number of rabbits to physiological salt-solution extracts of *Ascaris lumbricoides* from swine. The extracts were made by adding to salt solution pulverized material of entire worms, dried at room temperature shortly after they were removed from the host and washed in physiological salt solution, and extracting for a day or more at room temperature. After filtering the extracts they were preserved with a sufficient quantity of carbolic acid to make a 0.25 per cent. solution. Rabbits were injected intravenously and were bled about a week after the last injection. Small quantities of blood were obtained by severing the marginal ear vein under aseptic precautions. Larger quantities of blood were drawn directly from the heart under ether anesthesia.

Owing to the scarcity of material other than *Ascaris lumbricoides* from swine precipitating serum prepared by immunizing rabbits with extracts of that species only was used. The serum was tested against extracts of several species of ascarids as noted below. The extracts for the tests proper were prepared by adding a definite quantity of dried-worm material to a definite quantity of physiological salt solution and allowing it to extract for a day or longer in a refrigerator. When ready for use the extracts were filtered several times through ordinary filter paper until the filtrates were clear. In each series of tests similar quantities of coarsely pulverized material from each species were extracted in equal quantities of physiological salt solution at the same time and under identical conditions.

Following are the results of the first series of experiments:

The extracts employed in these tests were prepared on the basis of 100 mgm. of dry worm material to 2.5 c.c. of physiological salt solution. The precipitating serum which was used was rather weak.

Five drops of serum were added to tubes containing, respectively, five drops of extract of the following species: *Ascaris lumbricoides* (from swine), *Ascaris equorum, Belascaris marginata,* Toxascaris species (from a wild cat), *Ascaridia maculosa.*

The tube containing an extract of *Ascaris lumbricoides* showed a heavy precipitate in about 20 minutes. The contents of the tube containing an extract of *Ascaris equorum* showed marked clouding two hours after the serum had been added. This was followed by the

settling of a precipitate. The bulk of the precipitate was considerably less than that which settled in the tube containing an extract of *Ascaris lumbricoides*. The contents of the tubes containing extracts of Belascaris and Toxascaris were found to show cloudiness at about the same time, approximately two hours after the serum had been added. The amount of the precipitates formed in these tubes was smaller than that formed in the tube containing the extract of *Ascaris equorum*. The tube containing the extract of *Ascaridia maculosa* remained clear for about four hours during which it was kept under observation. An examination of the contents of the tube the following day showed a very light precipitate, much smaller in amount than those present in the tubes containing extracts of Belascaris and Toxascaris.

These experiments were repeated by using larger quantities of fluids, namely, 25 drops of extracts and 10 drops of serum. After adding the serum the tubes were placed in an incubator at a temperature of 37° C. After 15 minutes' incubation the tube containing the extract of *Ascaris lumbricoides* showed a marked precipitate. The tubes containing extracts of the other species were clear. After being taken out of the incubator the tubes were kept under observation over an hour, but no precipitates developed. The following day precipitates were found in all tubes. Judged by the quantity of precipitate present the tubes ranged in the following descending order: *Ascaris lumbricoides, Ascaris equorum,* Toxascaris, Belascaris and Ascaridia. The tube containing an extract of Ascaridia showed but a slight precipitate.

Additional experiments were carried out with serum diluted in physiological salt solution. Five drops of a 50 per cent. dilution of serum added to five drops of extract of each species referred to above yielded practically the same results as those obtained with pure serum except that precipitates were not noted in the tubes containing extracts of *Ascaris equorum,* Belascaris, Toxascaris and Ascaridia during the period that they were kept under observation (about four hours), while the contents of the tube containing an extract of *Ascaris lumbricoides* became cloudy in about 30 minutes and showed a marked precipitate 30 minutes later. An examination of the tubes the following day showed the presence of precipitates in all tubes except in that containing an extract of Ascaridia. The bulk of precipitate was greatest in the tube containing an extract of *Ascaris lumbricoides*. The tube containing an extract of *Ascaris equorum* was next in order, while that containing an extract of Belascaris showed the smallest quantity of precipitate. Five drops of a 30 per cent. dilution of serum added to five drops of extract of *Ascaris lumbricoides* caused the appearance of cloudiness followed by the formation of a precipitate in less than an hour. Extracts of other ascarids were not tested with this dilution of serum.

Further tests were made primarily with a view of obtaining more data on the differences in the degree and rate of reaction between extracts of *Ascaris lumbricoides* and *Ascaris equorum* by using similar extracts of the two species with equal quantities of serum. The results were uniformly the same, namely, a heavier and more rapidly appearing precipitate in tubes containing extracts of *Ascaris lumbricoides* than in those containing extracts of *Ascaris equorum.*

Each experiment and series of experiments was controlled as follows:

1. Extract of parasite plus a few drops of salt solution.
2. Precipitating serum plus a few drops of salt solution.
3. Normal serum plus a few drops of extract tested.

Unless the controls remained clear the results of the test or of the series of tests were disregarded. As a control on the general specificity of the test for ascarids, precipitating serum was tested against an extract of an unrelated form, namely, *Dictyocaulus filaria,* with negative results.

Inasmuch as the experiments which have just been summarized showed quite conclusively that extracts of the two species of the same genus, namely, *Ascaris equorum* and *Ascaris lumbricoides,* can be easily differentiated by the precipitin test the writer carried out a series of experiments at a later date to determine whether extracts of *Ascaris lumbricoides* from man can be differentiated by the same test from similar extracts of *Ascaris lumbricoides* from the hog. As a control on extracts of these forms an extract of *Ascaris equorum* was tested at the same time. The three extracts were prepared in a similar way, namely, by adding 0.3 gm. of coarsely powdered worm material from each host to 5 c.c. of physiological salt solution and allowing the mixtures to remain in a refrigerator for three days. The precipitating serum used in these tests was stronger than that used in the preceding experiments. The extracts were therefore diluted before being tested, since in the concentrated state differences between the rate of reaction of extracts of *Ascaris lumbricoides* and *Ascaris equorum* were lost.

The extracts were diluted from three to five times with physiological salt solution and 10 drops of extract were tested against 1 and 2 drops of serum, respectively. The tube containing an extract of *Ascaris equorum* did not show any precipitate until at least an hour after the addition of the serum, whereas the tubes containing extracts of *Ascaris lumbricoides* from the two hosts showed precipitates in a few minutes. No differences could be detected in the rate of the appearance of these precipitates, but as a rule the precipitates in the tubes containing extracts of *Ascaris lumbricoides* from swine were somewhat heavier than those in the tubes containing extracts of *Ascaris*

lumbricoides from man. It is doubtful, however, whether that fact has any significance in view of the rather crude manner in which the writer was obliged to prepare his extracts. Since material of *Ascaris lumbricoides* from man was scarce it was necessary to weigh out small quantities which were extracted in correspondingly small quantities of salt solution. It is possible that certain parts of the worm are more soluble in salt solution than others, so that when material from one or two specimens is used the quantity of extract obtained is less than when a similar quantity by weight is extracted from fragments of many specimens. Probably a more accurate method of performing the test would be to use the coelomic fluid of the worms instead of salt-solution extracts. It is expected that as soon as fresh material of *Ascaris lumbricoides* from man is available additional experiments will be undertaken to secure further data on that point.

These experiments were repeated by using more dilute extracts. Thus a dilution of each extract made by adding one part of the extract to nine parts of physiological salt solution and testing 10 drops of the diluted extract against 1 and 2 drops of serum, respectively, yielded the following results: After one hour the contents of the tubes containing extracts of *Ascaris lumbricoides* from the two hosts became cloudy, whereas the tube containing an extract of *Ascaris equorum* was perfectly clear. An examination of these tubes on the following day showed marked precipitates in those containing extracts of *Ascaris lumbricoides* from the two hosts and a slight precipitate in the tube containing an extract of *Ascaris equorum*. A still greater dilution of the extract, namely, 19 parts of salt solution to one part of extract yielded similar results; that is, the tubes containing extracts of *Ascaris lumbricoides* from the two hosts showed precipitates in about three hours, whereas the tube containing an extract of *Ascaris equorum* did not show a precipitate in eighteen hours.

The precipitating serum used in the above-mentioned series of experiments was tested against extracts of Toxascaris species and *Strongylus vulgaris* by adding equal quantities of serum to each extract. Inasmuch as the worm material which was extracted for these experiments was small in bulk the extracts were rather dilute. No precipitate appeared in the tube containing an extract of Strongylus after twenty hours. A very slight precipitate was seen in the tube containing an extract of Toxascaris after a similar period. An extract of *Ascaris lumbricoides* of approximately the same strength as those of the two parasites referred to above, plus an equal quantity of serum, showed a well-marked precipitate about an hour after the serum had been added.

All experiments in this series were controlled as has already been noted elsewhere in this paper.

Summarizing the results of the experiments concerning the relationship of the species of ascarids considered in the foregoing pages, it may be stated that the results of the precipitin tests correspond to the known zoological relationships of these parasites. The differences in the degree and rate of reaction between extracts of two species of the same genus are less than those between extracts of different genera. The slight reactions obtained with extracts of Ascaridia are decidedly significant in view of the fact that that genus is more distantly related to the genus Ascaris than are the genera Belascaris and Toxascaris. The two latter genera are included with Ascaris in the family Ascaridae, whereas the genus Ascaridia belongs to the family Heterakidae. These two families are included in the same superfamily, namely, Ascaroidea. No less significant is the failure to obtain any precipitates with extracts of Dictyocaulus and Strongylus, genera belonging to the superfamily Strongyloidea.

EXPERIMENTS WITH THE ANAPHYLACTIC TEST

The experiments with the precipitin test were supplemented by another series of immunologic tests, namely, by the anaphylactic reaction. The latter is based on the fact that an animal that has received an injection of protein material develops after a certain period a condition of hypersusceptibility to the protein or proteins in question, or, in other words, becomes sensitized to the protein or proteins. A reinjection of material similar to that used in the sensitizing injection calls forth a series of more or less grave symptoms which frequently terminate in death. The anaphylactic reaction is independent of the toxicity of the material injected, since it may be produced by substances that are nontoxic to normal animals.

Without describing in detail the exact response observed by the writer in guinea-pigs sensitized to very small quantities of extracts of various ascarids and reinjected after a period of incubation of two weeks or longer with extracts of the same species as that used for the sensitizing injection and with extracts of related species, the results of several series of experiments involving eighteen guinea-pigs will be summarized briefly. It is important to state in this connection that as a result of the work conducted by various members of this laboratory during the past two years, it was found that guinea-pigs are very tolerant of rather heavy single injections of the body fluids of *Ascaris lumbricoides*. These observations are in harmony with a considerable amount of published data on the effects of the body fluids of ascarids on various animals. The reactions observed by the present writer were considered to be, therefore, anaphylactic reactions and not reactions to any toxic constituents which the fluids of these parasites may contain.

For purpose of convenience the reactions will be referred to as follows:

Mild.—General body tremor, rapid breathing, muscular twitching, scratching of face, etc.

Marked.—In addition to the symptoms above, weakness in legs, frequent defecation and urination, tendency to fall down, etc.

Severe.—In addition to above symptoms, general paralysis.

The results of the experiments follow:

Series A. Six guinea-pigs were sensitized to a salt-solution extract of *Ascaris lumbricoides* from swine by a subcutaneous injection of 0.2 c.c. of an extract made by adding 0.5 gm. of powdered worm material to 15 c.c. of physiological salt solution and allowing the powder to extract for about two hours at room temperature. Fourteen to fifteen days later the animals were reinjected intraperitoneally with 2 c.c. of more concentrated extracts than that used for the sensitizing injection. The results follow:

No. 1. Reinjected with an extract of *Ascaris lumbricoides* from swine. Reaction mild.

No. 2. Reinjected as No. 1. Reaction mild.

No. 3. Reinjected with an extract of *Ascaris lumbricoides* from man. Reaction mild.

No. 4. Reinjected with an extract of Belascaris. No reaction.

No. 5. Reinjected with an extract of *Ascaridia maculosa.* No reaction.

No. 6. Reinjected with an extract of *Ascaris equorum.* No reaction.

Series B. The guinea-pigs used in this series were sensitized by subcutaneous injection of 0.5 c.c. of salt-solution extract of *Ascaris equorum* of about the same concentration as that used in sensitizing the animals of series A. Twelve days after the sensitizing injection the guinea-pigs were reinjected with 2 c.c. of more concentrated extracts as follows:

No. 7. Reinjected with extract of *Ascaris equorum.* Severe reaction.

No. 8. Reinjected with an extract of *Ascaris lumbricoides* (from swine). Marked reaction.

No. 9. Reinjected with an extract of Belascaris. Slight reaction.

Additional experiments were performed as follows:

No. 10. Sensitized to an extract of *Ascaris lumbricoides* (from man). Reinjected twelve days later with an extract of *Ascaris equorum.* Mild reaction.

No. 11. Sensitized to an extract of Belascaris and reinjected twelve days later with an extract of *Ascaris lumbricoides* (from swine). Reaction mild.

No. 12. Sensitized as No. 11 and reinjected thirteen days later with an extract of *Ascaris lumbricoides* (from man). Reaction mild.

No. 13. Sensitized as No. 11. Reinjected with an extract of Toxascaris thirteen days later. No reaction.

No. 14. Sensitized to an extract of *Ascaris lumbricoides* (from man). Reinjected with an extract of *Ascaris lumbricoides* (from swine) twelve days later. Mild reaction.

Series C. The guinea-pigs used in this series were sensitized to an extract of *Ascaris lumbricoides* (from swine). The extract employed for the sensitizing injections was prepared in a similar manner as that described for Series A. From 0.2 to 0.5 c.c. were used in the sensitizing injection which was given subcutaneously. The animals were reinjected eighteen days later with 1 c.c. of concentrated extracts as follows:

No. 15. Reinjected with an extract of *Ascaris equorum*. Severe reaction.
No. 16. Reinjected with an extract of *Ascaris equorum*. Severe reaction.
No. 17. Reinjected with an extract of *Ascaris lumbricoides* from swine. Fatal, death occurring forty minutes after the injection.
No. 18. Reinjected as No. 17. Mild reaction.

The results of experiments with the anaphylactic reaction are not so constant as the results of the precipitin tests, due, no doubt, to the fact that the latter take place in test tubes, whereas the former take place in living animals. Furthermore, the number of animals used is scarcely sufficient to justify any definite conclusions. In a general way, however, more marked reactions were obtained when a guinea-pig was reinjected with an extract of the same species as that used for the sensitizing injection than when it was reinjected with an extract of a related species. It is interesting to observe, also, that the guinea-pigs in series A, which were evidently only slightly sensitized to ascarid extracts, reacted to extracts of *Ascaris lumbricoides* from the two hosts in practically the same way, but that those that were reinjected with extracts of other species gave no reaction.

Attempts were also made to test the series of extracts of ascarids by means of the complement-fixation reaction, but in view of the fact that the extracts employed exhibited a marked tendency to yield non-specific complement fixation and that rabbit serum frequently exhibits anticomplementary properties, that phase of the work was temporarily abandoned.

SUMMARY AND CONCLUSIONS

1. The blood serum of rabbits immunized to salt-solution extract of *Ascaris lumbricoides* (from swine) causes the formation of precipitates when added to salt solution extracts of various ascarids (Ascaris, Belascaris, Toxascaris, Ascaridia). The precipitin reaction as applied to extracts of these parasites is therefore a group reaction.

2. By the use of proper dilutions heavier and more rapidly appearing precipitates are produced when rabbit serum immunized against *Ascaris lumbricoides* is added to salt solution extracts of *Ascaris lumbricoides* than when it is added to similar extracts of other ascarids. Extracts of species of the same genus (*Ascaris lumbricoides* and *Ascaris equorum*) show less difference in that respect than extracts of worms belonging to different genera (Ascaris, Belascaris, Toxascaris, Ascaridia). The results of the precipitin tests correspond, therefore, to the zoological relationships of these parasites.

3. Extracts of *Ascaris lumbricoides* from man do not appear to be distinguishable from extracts of *Ascaris lumbricoides* from swine so far as the results of the precipitin test are concerned. Apparently, the forms from the two hosts are biochemically as well as morphologically indistinguishable.

4. Small quantities of precipitating serum sufficient to cause the formation of precipitates in salt-solution extracts of ascarids failed to produce precipitates in similar extracts of unrelated nematodes (Dictyocaulus, Strongylus).

5. The results obtained by means of the anaphylactic test appear to be in a general way in agreement with the results of the precipitin test, although a considerable degree of variation was noted as regards the reactions of guinea-pigs to injections of similar extracts. Definite conclusions from the experiments on anaphylaxis are not justified in view of the limited number of experiments.

Literature Cited

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